

Evaluation of Cardiac Involvement During Dengue Viral Infection

Carlos Henrique Miranda,¹ Marcos de Carvalho Borges,¹ Alessandra Kimie Matsuno,² Fernando Crivelenti Vilar,¹ Luís Gustavo Gali,¹ Gustavo Jardim Volpe,¹ André Schmidt,¹ Antônio Pazin-Filho,¹ Fernanda Miquelitto Figueira da Silva,¹ Luiza Antunes de Castro-Jorge,¹ Mayra Fernanda de Oliveira,¹ Fabiano Saggioro,³ Rosecelis Brasil Martines,⁴ and Benedito Antônio Lopes da Fonseca¹

¹Department of Internal Medicine, ²Department of Pediatrics, and ³Department of Pathology, University of São Paulo Medical School at Ribeirão Preto; and ⁴Pathology Center, Adolfo Lutz Institute, São Paulo, Brazil

Background. Dengue is a disease whose clinical manifestations range from asymptomatic infections to a severe disease. There have been some previous reports of myocardial involvement in dengue, but this association has not been completely established.

Methods. From January to July of 2011, patients hospitalized with dengue, confirmed through dengue non-structural protein 1 and/or immunoglobulin M detection, were included in this study and troponin I and N terminal fragment of B-type natriuretic peptide levels were determined. Patients with abnormal biomarkers underwent echocardiography and when any abnormality was detected, they underwent cardiac magnetic resonance imaging.

Results. Eighty-one patients were evaluated and 12 patients (15%) presented with elevated biomarker levels. Compared to controls, they had higher leukocyte ($P < .001$) and platelet counts ($P = .005$); higher C-reactive protein ($P = .02$), and a lower viral load ($P = .03$). There was no difference according to clinical dengue classification; dengue hemorrhagic fever/dengue shock syndrome severity; duration of symptoms; or prevalence of secondary infection between the 2 groups. Two patients died secondary to cardiogenic shock before imaging studies. Necroscopic findings were compatible to myocarditis in both, and immunohistochemistry for dengue virus showed increased staining on mononuclear cells located in the myocardial tissue. Of the 10 patients who underwent echocardiography, depressed left ventricular ejection fraction (LVEF) was identified in 1, left ventricular segmental abnormalities with preserved LVEF in 2, and an important pericardial effusion with tamponade in another. Cardiac involvement was confirmed by CMR in these 4 patients.

Conclusions. Dengue viruses were shown to cause cardiac disease with clinical manifestations ranging from mild elevation of biomarkers to myocarditis and/or pericarditis.

Keywords. dengue; myocarditis; cardiac biomarkers; cardiogenic shock.

Dengue, a disease caused by any of the 4 dengue virus (DENV) serotypes (DENV-1, -2, -3, and -4), is the most important arthropod-borne viral disease in the world. Dengue is endemic to tropical and subtropical

regions of the world, where it represents an important public health problem. This disease has spread geographically to many previously unaffected areas and, with increasing global trade in recent years, physicians in temperate areas of the world are more likely to care for returning travelers with dengue infection [1].

Dengue clinical manifestations range from a mild, flulike syndrome to a more severe disease associated with plasma leakage, thrombocytopenia, hemorrhage, and/or shock. Although there are some reports of myocarditis complicating dengue infection, cardiac involvement in dengue has been poorly investigated [2, 3].

Because there is neither a vaccine nor an effective antiviral treatment available for dengue [4, 5], the purposes

Received 19 February 2013; accepted 6 June 2013; electronically published 19 June 2013.

Correspondence: Benedito Antonio Lopes da Fonseca, MD, PhD, Disciplina de Moléstias Infecciosas e Tropicais, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP 14049-900, Brasil (baldfons@fmrp.usp.br).

Clinical Infectious Diseases 2013;57(6):812-9

© The Author 2013. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/cit403

of this study were to prospectively investigate the cardiac involvement in patients with dengue infection and to better evaluate its clinical and pathological manifestations.

PATIENTS AND METHODS

Patients

All dengue patients admitted to the emergency department of our hospital between January and July 2011 were included in the study. Dengue suspected cases were defined according to the World Health Organization (WHO) 2009 criteria associated with a rapid test detection of nonstructural protein 1 (NS1) and/or immunoglobulin M (IgM) antibody on patients' serum [6].

The local research ethics committee approved the study. All patients gave written informed consent to participate; for those patients in critical condition or aged <18 years, an informed consent was obtained from their relatives or legal guardians.

Study Design

General laboratory tests such as complete blood count, C-reactive protein, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine levels were collected at hospital admission for all patients and these results were used in this study. Dengue was classified as either dengue fever (DF) or dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) according to the WHO 1997 classification [7]. IgG/IgM capture enzyme-linked immunosorbent assay (ELISA) (PanBio Diagnostics, Brisbane, Australia) and reverse transcription polymerase chain reaction (RT-PCR) were used to detect dengue virus infection.

A rapid test (SD BIOLINE Dengue Duo, Standard Diagnostic Inc, Korea) was used to detect NS1 viral protein and dengue-specific IgM and IgG antibody. An IgG/IgM capture ELISA was then performed according to the manufacturer's instructions [8–10]. Viral RNA was extracted from serum samples using QIAamp Viral RNA kit (Qiagen, Inc, Valencia, California) according to the manufacturer's instructions. RT-PCR, used for dengue serotype determination, was carried out using the Qiagen One-Step RT-PCR kit as previously described [11]. Dengue viral load was determined by locally standardized protocol using QuantiTect Virus Kit (Qiagen, Germantown, Maryland).

Secondary infections were defined as the presence of positive RT-PCR and/or positive NS1 associated with IgG positivity during the acute phase (<7 days' disease duration) and an IgM/IgG ratio of <1.2 during the convalescent phase (≥ 7 days' disease duration) [6, 8].

Troponin I and N terminal fragment of B-type natriuretic peptide (NT-proBNP) levels were determined for all dengue patients at hospital admission. Troponin I was measured using an enzyme-linked fluorescent assay (VIDAS Troponin I Ultra, bioMérieux, France) and serum levels >0.01 ng/L were

considered abnormal (99th percentile of a reference control group). NT-proBNP levels were also measured by an enzyme-linked fluorescent assay (VIDAS NT-proBNP, bioMérieux, France) and were used to identify patients with suspected left ventricular dysfunction. NT-proBNP levels were considered altered according to the patient's age (>450 $\mu\text{g/L}$ for age <50 years, >900 $\mu\text{g/L}$ for age 50–75 years, and >1800 $\mu\text{g/L}$ for age >75 years). NT-proBNP levels <300 $\mu\text{g/L}$ were considered normal regardless of age [12].

All patients with an elevated troponin I and/or NT-proBNP levels underwent 2-dimensional echocardiography (ECO). A left ventricular ejection fraction (LVEF) of <50% was considered depressed.

If any abnormalities were detected in the ECO, at least 1 cardiac magnetic resonance imaging (CMR) scan was performed. An LVEF of <56% was considered abnormal. CMR myocardium characterization evaluated the 3 most important tissue parameters: myocardium edema through T2-weighted imaging; hyperemia and capillary leak due to regional vasodilation in areas with important inflammation through the myocardial early gadolinium enhancement; and delayed-hyperenhancement areas demonstrating irreversible myocardial injury [13]. ECO was performed up to 1 week after patient admission, and CMR was performed as soon as the patient had stable clinical condition.

Necroscopic examination was performed in both patients who did not survive, and a myocardial histologic examination included Masson's trichrome and hematoxylin and eosin staining. Conventional immunohistochemistry protocol used an antidengue polyclonal antibody produced in mice.

Statistical Analysis

Quantitative variables are reported as mean \pm SD and the categorical variables as frequency or percentage. Quantitative variables with non-Gaussian distribution were expressed as median and interquartile range (IQR; P_{25} – P_{75}). The difference between means was determined by Student *t* test in data with Gaussian distribution and Mann-Whitney test for data with non-Gaussian distribution. The association between categorical variables was analyzed by Fisher exact test. A *P* value of <.05 was considered to be significant in all analyses.

RESULTS

Baseline Characteristics

Clinical and laboratorial characteristics of all 81 dengue patients included in the study are shown in Table 1. Mean age of patients was 32 years (SD, 21 years; range, 4 months to 81 years). All patients presented with typical clinical manifestations of dengue and the median duration of symptoms at hospital admission was 4 days (IQR, 3–6). Fifty-four patients (67%) were classified as having dengue fever, 26 patients (32%) as DHF grade I–II, and

Table 1. Clinical and Laboratory Characteristics of Dengue Patients (N = 81)

Characteristics	No. (%)
Demographic	
Age, y	32.2 ± 21.7
Male	39 (48)
Symptoms	
Duration, d, median (interquartile range)	4 (3–6)
Fever	81 (100)
Myalgia	54 (67)
Headache	53 (65)
Extreme fatigue	43 (53)
Rash	37 (46)
Abdominal pain	24 (30)
Bleeding	20 (25)
Arthralgia	17 (20)
Diarrhea	16 (20)
Shock	3 (4)
Diagnostic tests (rapid test)	
NS1 antigen	65 (80)
IgM antibody	32 (39)
IgG antibody	21 (26)
Clinical classification	
Dengue fever	54 (67)
DHF/DSS	27 (33)
Grade I–II	26 (32)
Grade III–IV	1 (1)
Primary infection	51 (63)
Secondary infection	30 (37)
Laboratory findings (mean ± SD)	
Hemoglobin, g/dL	13.5 ± 1.8
Hematocrit, %	40.8 ± 5.6
Hematocrit >45%	15 (18)
White blood cell count, cells/mm ³	4907.4 ± 3770.0
Platelet count, cells/mm ³	117 543.2 ± 89 198.2
Platelet count <100,000/mm ³	41 (51)
Creatinine, mg/dL	0.8 ± 0.2
AST 3× upper limit, U/L	19 (23)
ALT 3× upper limit, U/L	14 (17)
Troponin I elevation	6 (7)
Troponin I, µg/L, abnormal range	0.03–3.54
NT-proBNP	
Normal range	59 (73)
Intermediate range	12 (15)
Abnormal range	10 (12)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; DHF/DSS, dengue hemorrhagic fever/dengue shock syndrome; IgG, immunoglobulin G; IgM, immunoglobulin M; NS1, nonstructural dengue viral protein 1; NT-proBNP, N terminal fragment of B-type natriuretic peptide; SD, standard deviation.

only 1 patient (1%) as DHF grade III–IV. Dengue virus infection was confirmed through detection of NS1 viral protein in 80% and specific IgM antibody in 39% of the patients. Primary and secondary dengue infection occurred in 51 (63%) and 30 (37%) patients, respectively. Laboratory examination revealed hemoconcentration in 18% of the patients; severe thrombocytopenia (platelets <100 000/mm³) in 51%; hepatic involvement in 17% and 23%, according to the ALT and AST elevation, respectively. Only 2 patients had abnormal creatinine level (>1.5 mg/dL). Bleeding occurred in 25% of the patients, usually epistaxis.

Cardiac Biomarkers

Twelve (15%) dengue patients showed increased levels of at least 1 cardiac biomarker, and 4 patients, of both. Troponin I level was increased in 6 (7%) patients, ranging from 0.03 µg/L to 3.54 µg/L, and NT-proBNP level was increased in 10 (12%) patients. However, in 12 (15%) additional patients, NT-proBNP levels reached an intermediate level, but as they were considered a “gray zone,” it was not possible to establish the diagnosis of heart failure. Thus, the imaging examination was not performed on these patients.

Comparison Between Patients With and Those Without Biomarker Elevation

Characteristics of patients with increased levels of troponin I and/or NT-proBNP and patients with normal levels are shown in Table 2. There was no significant difference in dengue classification, DHF/DSS severity, duration of symptoms, and prevalence of secondary dengue infection between these 2 groups.

Dengue patients with biomarker elevation had higher leukocyte (9350 ± 6125 cells/mm³ vs 4150 ± 2597 cells/mm³; *P* < .001) and platelet counts (188 166 ± 149 897 cells/mm³ vs 109 347 ± 72 024 cells/mm³; *P* = .005), as well as higher levels of C-reactive protein (6.7 ± 10 mg/L vs 1.3 ± 1.8 mg/L, *P* = .02) and creatinine (1.1 ± 0.8 mg/dL vs 0.8 ± 0.2 mg/dL; *P* = .03).

The DENV-1 serotype was identified in 12 (100%) patients with increased biomarkers and in 58 (84%) patients without increased biomarkers. A lower viral load was significantly observed in the group with elevation of the biomarkers compared to patients without biomarker elevation (4.74 ± 1.1 log₁₀ copies/mL vs 6.18 ± 2.2 log₁₀ copies/mL; *P* = .03).

Evaluation of the Patients With Biomarkers Elevation

Clinical, laboratory, and imaging characteristics of the 12 (15%) patients with elevated cardiac biomarkers are shown in Table 3. Eight (10%) patients presented with clinical manifestations suggestive of cardiac involvement, such as acute heart failure in 4 patients, chest pain in 3 patients, and hypotension and shock in 3 patients.

Table 2. Comparison Between Patients With and Those Without Cardiac Biomarkers Elevation

Characteristics	With Biomarker Elevation (n = 12)	Without Biomarker Elevation (n = 69)	PValue
Age	37 ± 25	31 ± 21	.42
Male sex	6 (50)	34 (49)	1.00
Dengue fever	9 (75)	45 (65)	.74
Dengue hemorrhagic fever	3 (25)	24 (35)	.74
Grade I–II	3 (25)	23 (33)	.74
Grade III–IV	0 (0)	1 (1)	1.00
Secondary infection	7 (58)	23 (33)	.11
Duration of symptoms			
Median, d	5	4	.36
Interquartile range	3–6	3–6	
NS1 antigen	8 (67)	57 (83)	.20
IgG antibody/ELISA	8 (67)	31 (45)	.21
IgM antibody/ELISA	10 (83)	36 (52)	.06
RT-PCR (DENV-1)	12 (100)	58 (84)	.20
Viral load, log ₁₀ copies/mL	4.74 ± 1.1	6.18 ± 2.2	.03
Hemoglobin, g/dL	13 ± 3	13 ± 2	.78
Hematocrit, %	41 ± 8	41 ± 5	.87
White blood cell count, cells/mm ³	9350 ± 6125	4150 ± 2597	<.001
Platelet count	188 166 ± 149 897	109 347 ± 72 024	.005
Troponin I levels	0.75 ± 1.2	0.01 ± 0.0	.003
NT-proBNP levels	1917 ± 3125	159 ± 275	<.001
C-reactive protein	6.7 ± 10	1.3 ± 1.8	.02
Creatinine	1.1 ± 0.8	0.8 ± 0.2	.03
Death	2 (17)	1 (1.2)	.05

Abbreviations: DENV-1, dengue virus serotype 1; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; NS1, nonstructural dengue viral protein 1; NT-proBNP, N terminal fragment of B-type natriuretic peptide; RT-PCR, reverse transcription polymerase chain reaction.

Two patients developed refractory cardiogenic shock and died before imaging examination could be performed. These patients were young (aged 26 and 29 years) and presented no comorbidities, as well as no evidence of other infections, bleeding, or hemoconcentration. Histopathological findings of the necroscopic examination showed marked interstitial edema with a diffuse inflammatory infiltrate composed of mononuclear cells, mainly macrophage-type cells in the myocardium (Figure 1A and 1B). Immunohistochemistry for dengue viruses in myocardial specimens showed marked staining on mononuclear cells and slight intracytoplasmic granular staining within some cardiomyocytes (Figure 2). There were no abnormalities in the coronary circulation or evidence for bacterial or fungal infection in any organs.

Among the other 10 patients, 4 showed alterations in ECO. Of them, 2 had abnormal wall motion with preserved LVEF showing mainly important apical hypokinesia, with an intracardiac thrombus in this region in one of them (Figure 1C); 1 patient had abnormal wall motion and depressed LVEF (Figure 1D) and another had an important pericardial effusion with tamponade signals but without myocardium involvement.

Cardiac involvement was confirmed by CMR in these 4 patients. In 3 patients, depressed LVEF associated with abnormal wall motion was observed. Tissue characterization on these patients showed hyperintense signal in T2 in 3 patients (Figure 1E), early enhancement in 1 patient, and late gadolinium enhancement in 3 patients. One patient had alteration in all 3 parameters of the tissue characterization; 1 patient had 2 parameters altered; and 2 patients had only 1 parameter altered. Out of these 2 last patients, one had only pericardium involvement with important late gadolinium enhancement in the pericardium, confirming the diagnosis of pericarditis, but without myocardium involvement (Figure 1F). The other patient had only the hyperintense signal in T2.

The pericardial effusion had an important hemodynamic impact on the patient presenting with this condition and a pericardiocentesis was performed to restore the patient's hemodynamic parameters. Dengue-specific IgG and IgM antibodies were detected in the pericardial fluid. Due to a high prevalence in our country, tuberculosis was also investigated in this case but resulted negative by both real-time PCR and culture to *Mycobacterium tuberculosis*.

DISCUSSION

The present study systematically investigated cardiac involvement in dengue patients by using sensitive and specific biomarkers associated to imaging methods, such as CMR, which is considered the gold standard for evaluation of myocarditis and pericarditis. Cardiac involvement was observed in 15% of patients requiring hospitalization, with clinical manifestation ranging from mild elevation of cardiac biomarkers to myocarditis and/or pericarditis and death.

Elevation of Biomarkers in Dengue Disease

To our knowledge, only 1 study evaluated the elevation of cardiac biomarkers during dengue virus infection. In Sri Lanka, Wichmann et al [14] showed that 25% of dengue patients presented with 1 or more elevated markers of myocardial injury, such as myoglobin, CK-MB, troponin T, NT-proBNP, and/or heart-type fatty acid binding protein levels (h-FABP). However, the majority of patients had elevation of myoglobin or CK-MB, which is a nonspecific biomarker of myocardial injury. Furthermore, only 1 patient (0.8%) had elevation of troponin and 25 patients (18.9%) had elevation of NT-proBNP; however,

Table 3. Characteristics of Patients With Elevated Level of Biomarkers (Troponin and/or NT-proBNP)

Patient Number	Demographic			Clinical			Laboratory					Echocardiography			Cardiac Magnetic Resonance						
	Age (y)	Sex (M/F)	Symptom Duration (days)	Classification	Cardiac Manifestation	Death (Y/N)	Troponin I	NT-proBNP	NS1	IgG/ELISA	IgM/ELISA	Hematocrit (%)	Platelet Counts	LVEF (%)	Abnormal Wall Motion (Y/N)	Pericardium Effusion (Y/N)	LVEF (%)	Abnormal Wall Motion (Y/N)	Hyperintense Signal in T2 (Y/N)	Early Gadolinium Enhancement (Y/N)	Late gadolinium enhancement
1	29	F	3	DF	Shock/HF	Y	1.28	1022	Y	Y	Y	41	47 000	(...)
2	26	M	3	DF	Shock	Y	0.69	1212	Y	N	N	30	308 000	(...)
3	26	M	16	DF	HF	N	3.54	533	N	Y	Y	45	447 000	54	Y	N	50	Y	Y	Y	Y
4	29	F	7	DF	HF	N	2.52	11 298	Y	Y	Y	48	156 000	34	Y	N	49	Y	Y	N	N
5	39	M	30	DF	CP	N	0.21	54	N	N	Y	51	349 000	57	Y	N	40	y	Y	N	Y
6	47	M	5	DF	Shock/HF	N	<0.01	1097	N	Y	Y	42	271 000	50	N	Y	58	N	N	N	Y ^a
7	42	F	3	DF	CP/HF	N	<0.01	543	Y	Y	Y	37	60 000	54	N	N	(...)
8	86	F	6	DF	N	N	<0.01	4135	N	Y	Y	39	41 000	55	N	N	(...)
9	3	M	5	DF	N	N	<0.01	872	Y	Y	Y	33	121 000	56	N	N	(...)
10	4	F	3	DHF	N	N	<0.01	910	Y	N	N	34	362 000	52	N	N	(...)
11	75	M	2	DHF	N	N	0.03	752	Y	Y	Y	56	50 000	67	N	N	(...)
12	36	F	5	DHF	CP/HF	N	<0.01	578	Y	N	Y	31	46 000	75	N	N	(...)

Abbreviations: CP, chest pain; DHF, dengue hemorrhagic fever; DF, dengue fever; ELISA, enzyme-linked immunosorbent assay; HF, heart failure; IgG, immunoglobulin G; IgM, immunoglobulin M; LVEF, left ventricular ejection fraction; NS1, nonstructural dengue viral protein 1; NT-proBNP, N terminal fragment of B-type natriuretic peptide.

^a Only localized in the pericardium.

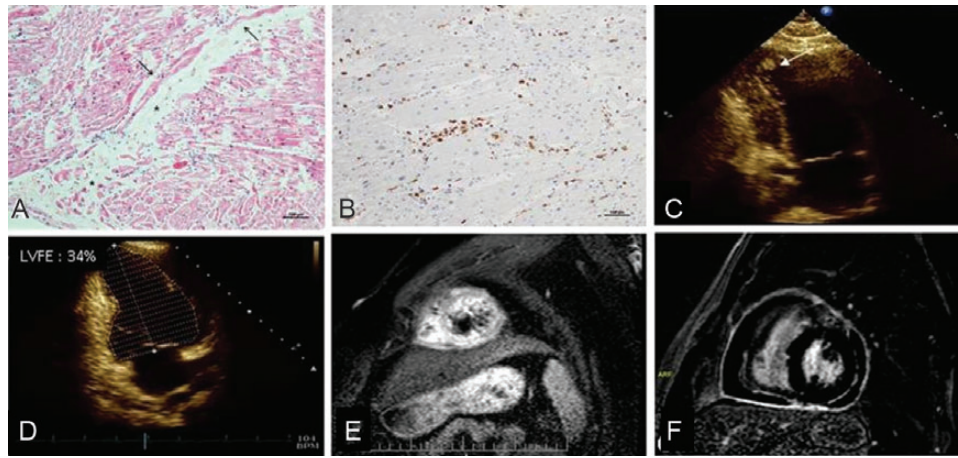


Figure 1. Figure depicting histopathological and radiological findings of patients with dengue fever and elevated biomarkers. *A*, Studies showing important interstitial edema and an inflammatory infiltrate in the myocardium composed of mononuclear cells (*). The myocytes are overstretched and wavy in small clusters (arrows). Hematoxylin and eosin staining ($\times 100$). *B*, Cell population is composed mainly of CD68-positive macrophage-type cells in the mononuclear inflammatory infiltrate observed in the myocardium. Anti-CD68 immunostaining ($\times 100$). *C*, Echocardiography showing important apical hypokinesia with formation of intracardiac thrombus (arrow) in this region (patient 5). *D*, Echocardiography showing important depression in left ventricular ejection fraction in the acute phase with late recovery (patient 4). *E*, Cardiac magnetic resonance imaging (CMR) showing hyperintense signal in T2 in the apical region compatible with important edema (patient 5). *F*, CMR showing pericardium effusion with late gadolinium enhancement in pericardium (patient 6).

distinct NT-proBNP cutoff levels were used [14]. In our study, we observed troponin I elevation in 6 (7%) patients and important elevation of NT-proBNP in 10 (10%) patients.

Patients with elevated biomarkers showed a more intense inflammatory activity confirmed by a higher leukocyte count and C-reactive protein levels. In agreement with this inflammatory activity, a lower viral load was observed in this group of patients, suggesting there is no association between viral load and cardiac injury. Thus, it is possible that the inflammatory activity secondary to dengue virus has a pivotal role in the pathophysiology of the cardiac disease, and the myocardial injury could be an immune-mediated event.

Although dengue complications are described to be more frequent during secondary infection, in our study, cardiac involvement was not more prevalent in dengue patients with secondary infection. Because there was no significant difference in clinical dengue classification and DHF severity, and the only patient classified as DFH grade III–IV had no elevation of cardiac biomarkers, we believe that the severity of dengue presentation does not explain the impairment of the cardiac function in these patients.

Heart Failure and Cardiogenic Shock

There are few reports of acute heart failure during dengue virus infection. In an evaluation of 17 dengue patients with radionuclide ventriculography, Wali et al showed that 7 patients had an ejection fraction of $<40\%$ and 12 had global hypokinesia, and that, after 3 weeks of follow-up, all alterations had returned to

normal [15]. In another report of 102 children with DHF, 10 patients had acute myocarditis requiring use of inotropic drugs due to acute heart failure [16]. In our study, 4 patients had symptoms of acute heart failure. Depressed LVEF was confirmed in 1 and 3 patients with ECO and CMR, respectively. Abnormality of wall motion was observed in 3 patients with both ECO and CMR.

Three patients developed shock and 2 of these patients died. Dengue can cause a severe form of circulatory shock due to an important plasma leakage associated with bleeding and hemoconcentration [2]. As these 3 patients had normal hemoglobin and hematocrit levels and no signal of bleeding or hemoconcentration, this possible complication was ruled out, and the cardiac involvement was considered the etiology of the shock. There was no evidence of bacterial or fungal infection in either blood cultures or any organs ruling out septic shock as the cause for this hemodynamic involvement. Due to these reasons, we believe that cardiogenic shock was the most probable cause of shock in these patients. Our data are unique in several aspects as the majority of our patients were adults, including the 2 who died; that there are few descriptions of fulminant myocarditis in dengue patients resulting in refractory cardiogenic shock due to dengue infection; and it is the first study to use CMR to study the cardiac involvement in dengue [17–19].

Necroscopic Findings and Pathophysiological Mechanisms

Weerakoon et al [20] performed autopsies in 5 patients (3 females and 2 males) who died due to dengue complications

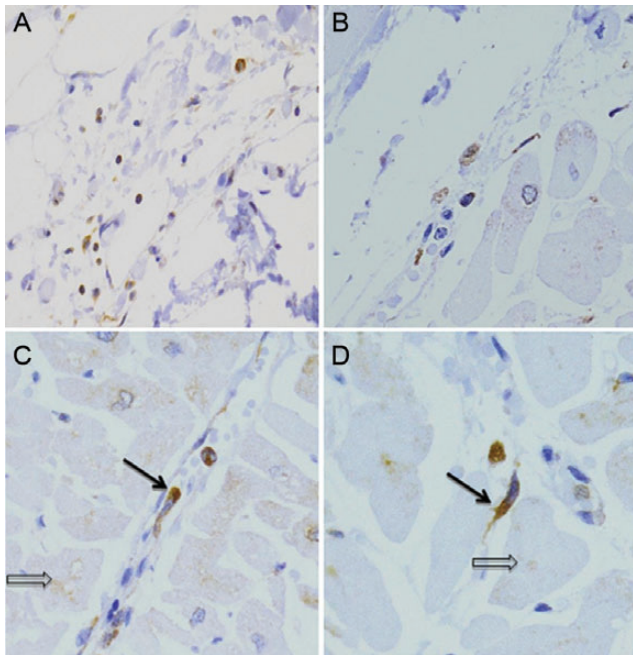


Figure 2. Photomicrographs of myocardium sections of patients who died from dengue (original magnification: A, $\times 100$; B, $\times 200$; C and D, $\times 400$). A polyclonal antidengue antibody detected the presence of dengue virus on tissue specimens. Sections show dengue-specific immunostaining of inflammatory cells with viral antigens (black arrows). A less intense staining is observed in cardiomyocytes (open arrows).

and showed histopathological evidence of myocarditis. The main histological findings of the heart were interstitial edema with inflammatory cell infiltration and necrosis of myocardial fibers and, in one case, evidence of pericarditis. These histological findings were similar to those observed in our study.

In association with the findings of intense infection of mononuclear cells observed on myocardial immunohistochemistry, the main mechanism of myocardial damage in dengue could be the release of inflammatory mediators and not only the direct action of the virus on cardiomyocytes [19, 21].

Salgado et al [22], using immunofluorescence confocal microscopy in heart tissue, reported that myotubes were infected by dengue virus in 1 child with fatal DHF, although the myocardium sections appeared morphologically normal, with minimal cellular infiltrates. However, clinical characterization of myocarditis in this case was not complete.

Cardiac Magnetic Resonance Imaging Findings

In our study, of the 4 patients who underwent CMR, one of them had only pericardial involvement, with important late gadolinium enhancement in the pericardium, confirming the diagnosis of pericarditis. This patient presented with clinical findings of cardiac tamponade signs that resolved completely after pericardiocentesis.

In the 3 other patients, all 3 CMR parameters of tissue characterization of the myocardial damage were observed in 1 patient, 2 parameter changes in patient, and only 1 parameter change in another patient. For myocarditis diagnosis, changes in 1 or 2 parameters of cardiac tissue involvement have accuracy of 67%–75% and 73%–85%, and positive predictive values of 68% and 95%, respectively. Therefore, it is well recognized that the presence of at least 2 positive criteria defines the CMR study as positive for myocarditis [13].

In our study, only 1 patient had < 2 positive CMR criteria for myocarditis. This patient developed cardiogenic shock needing mechanical ventilation and dobutamine infusion. The ECO performed in the first week showed an important depression of LVEF. Due to the severity of cardiogenic shock, CMR was performed later in the disease when left ventricular function had already improved.

Limitations

This study has some methodological limitations. First, we used the cardiac biomarker elevation for patient screening in order to select the candidates to perform cardiac imaging. This could underestimate the real prevalence of dengue myocarditis, as a large percentage of patients can have myocarditis without biomarker elevation; for example, troponin I has high specificity (89%) but limited sensitivity (34%) in the diagnosis of myocarditis [21]. Furthermore, NT-proBNP has not been specifically tested for myocarditis diagnosis, but for acute heart failure diagnosis, it has a sensitivity and specificity of 90% and 84%, respectively, when using the cutoffs used in this study [12]. Second, in dengue patients with elevated biomarkers, ECO examination was performed as soon as possible, but CMR was only done when any abnormalities were detected in the ECO. CMR is more sensitive than ECO for myocarditis diagnosis, allowing one to evaluate tissue changes, whereas ECO visualizes only functional abnormalities and pericardial effusion [13]. The diagnostic value of ECO is limited by the fact that many patients with less severe myocarditis have a normal ECO [21]. If CMR had been performed in all patients, we would have probably observed more structural alterations, but due to its costs and the need of a hemodynamic stable patient, we decided to perform ECO first. Fourth, it was not possible to completely exclude coinfections, such as enteroviruses, in all dengue patients. However, we feel that is not the case as, in our study, DENV-1 was detected in every patient with evidence of myocardial injury, and also, in most of them, the NS1 protein was readily detected, showing evidence for an acute dengue infection. Finally, all patients in our study required hospitalization and represent a selected dengue population with more severe clinical manifestations, but that should not be a problem of our study as these are the patients who will more likely need medical attention.

In conclusion, this study shows that the heart can be affected during DENV infection in a considerable percentage of patients needing hospital admission. Clinical manifestations range from mild elevation of biomarkers to myocarditis and/or pericarditis. These cardiac complications might be underdiagnosed in clinical practice and can contribute to the mortality observed in dengue.

Notes

Acknowledgments. We gratefully acknowledge the technical assistance in the echocardiography examinations of Ana Marta Antunes Salgado Gali, Anelise Aurelio Bettarello, and Edgard Camilo de Oliveira Filho.

Financial support. This work was supported by the São Paulo Research Foundation (FAPESP; grant numbers 2009/14449-4 and 2011/22514-0).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Simmons CP, Farrar JJ, Nguyen V, Wills B. Dengue. *N Engl J Med* **2012**; 366:1423–32.
2. da Fonseca BA, Fonseca SN. Dengue virus infections. *Curr Opin Pediatr* **2002**; 14:67–71.
3. Gulati S, Maheshwari A. Atypical manifestations of dengue. *Trop Med Int Health* **2007**; 12:1087–95.
4. Noble CG, Chen YL, Dong H, et al. Strategies for development of dengue virus inhibitors. *Antiviral Res* **2010**; 85:450–62.
5. Valero N, Levy A. Future prospects in developing of dengue vaccines. *Invest Clin* **2008**; 49:135–8.
6. World Health Organization. Dengue: Guidelines for treatment, prevention and control. Geneva, Switzerland: WHO, **2009**.
7. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva, Switzerland: WHO, **1997**.
8. Castro-Jorge LA, Machado PR, Favero CA, et al. Clinical evaluation of the NS1 antigen-capture ELISA for early diagnosis of dengue virus infection in Brazil. *J Med Virol* **2010**; 82:1400–5.
9. Chuansumrit A, Chaiyaratana W, Pongthanapisith V, Tangnararatchakit K, Lertwongrath S, Yoksan S. The use of dengue nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. *Pediatr Infect Dis J* **2008**; 27:43–8.
10. Groen J, Koraka P, Velzing J, Copra C, Osterhaus AD. Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G antibodies. *Clin Diagn Lab Immunol* **2000**; 7:867–71.
11. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* **1992**; 30:545–51.
12. Januzzi JL, van Kimmenade R, Lainchbury J, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. *Eur Heart J* **2006**; 27:330–7.
13. Friedrich MG, Sechtem U, Schulz-Menger J, et al. Cardiovascular magnetic resonance in myocarditis: a JACC white paper. *J Am Coll Cardiol* **2009**; 53:1475–87.
14. Wichmann D, Kularatne S, Ehrhardt S, et al. Cardiac involvement in dengue virus infections during the 2004/2005 dengue fever season in Sri Lanka. *Southeast Asian J Trop Med Public Health* **2009**; 40:727–30.
15. Wali JP, Biswas A, Chandra S, et al. Cardiac involvement in dengue haemorrhagic fever. *Int J Cardiol* **1998**; 64:31–6.
16. Salgado DM, Panqueba CA, Castro D, Vega R, Rodriguez JA. Myocarditis in children affected by dengue hemorrhagic fever in a teaching hospital in Colombia. *Rev Salud Publica (Bogota)* **2009**; 11:591–600.
17. Lee CH, Teo C, Low AF. Fulminant dengue myocarditis masquerading as acute myocardial infarction. *Int J Cardiol* **2009**; 136:e69–71.
18. Thomas L, Brouste Y, Najioullah F, et al. Prospective and descriptive study of adult dengue cases in an emergency department, in Martinique. *Med Mal Infect* **2010**; 40:480–9.
19. Miranda CH, Borges MC, Schmidt A, Pazin-Filho A, Rossi MA, Ramos SG, et al. A case presentation of a fatal dengue myocarditis showing evidence for dengue virus-induced lesion. *Eur Heart J* **2013**; 2: 127–30.
20. Weerakoon KG, Kularatne SA, Edussuriya DH, et al. Histopathological diagnosis of myocarditis in a dengue outbreak in Sri Lanka, 2009. *BMC Res Notes* **2011**; 4:268.
21. Cooper LT Jr. Myocarditis. *N Engl J Med* **2009**; 360:1526–38.
22. Salgado DM, Eltit JM, Mansfield K, et al. Heart and skeletal muscle are targets of dengue virus infection. *Pediatr Infect Dis J* **2010**; 29: 238–42.